produced a-10 mV shift in the availability curve, slowed recovery from inactivation and increased the late Na current ($I_{Na\text{-}L}$). These changes were prevented by inclusion of the CaMKII inhibitor peptide, AIP in the pipette or partially prevented by exposure to the PKA inhibitor PKI. $Na_V1.5$-S571A, $Na_V1.5$-S526H and $Na_V1.5$-S528A channels eliminated the CaMKII induced shift in inactivation, slowing of recovery and increase in $I_{Na\text{-}L}$ S571D or S528D mimicked the effects of phosphorylation. In $Na_V1.5$-S1969A channels exposure to CAMKII increases $I_{Na\text{-}L}$ compared to $Na_V1.5$-WT with a further increase in $I_{Na\text{-}L}$ in the presence of CaMKII; however, the steady state inactivation curve is not shifted. These data suggest the presence of functional phosphorylation sites in the CT and the interaction of CaMKII and PKA in the I-II linker. The cross talk between CaMKII and PKA modulation of the channel may have important implications for electrophysiological properties of the heart.

2917-Pos Board B347
Recruitment of Calmodulin to the Tail of the Voltage-Gated Sodium Channel Nav1.2
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Voltage-gated sodium channels (NaV) found in excitable cells are responsible for the rising phase of action potentials. These multi-domain transmembrane proteins are regulated by calmodulin (CaM), a highly conserved eukaryotic protein that mediates many calcium-triggered signaling events. Inactivation of sodium channels depends on CaM-mediated feedback during repolarization. In the neuronal sodium channel NaV1.2, CaM binds at least two well-separated sites: an intracellular ''inactivation'' loop between domains DIII and DIV, and an IQ motif [IQRAYRRYLLK] in the cytosolic C-terminal tail. The IQ motif is hypothesized to recruit calcium-free (apo) CaM, making it available to move to the III-IV linker after an influx of calcium. Despite a high degree of sequence identity, the equilibrium constants for CaM binding to nine human NaV IQ motifs span more than 3 orders of magnitude. Apo CaM binds to the NaV1.2 IQ motif with a dissociation constant (Kd) of ~6 nM, while the Kd for binding the NaV1.9 IQ motif is ~ 4 μM. Mutational analysis within the IQ motif has not been sufficient to explain the full range of CaM-binding affinities observed for human NaV sequences. Thus, we hypothesized that isoform-specific differences in upstream sequences were making energetically significant contributions to the free energy of binding CaM to NaV1.2. The roles of these residues are being investigated by monitoring CaM binding to biosensors containing mutant sequences of sodium channels bracketed by auto-fluorescent proteins YFP and CFP. Residue-specific information obtained by NMR will provide structural insight into the contributions of residues in the binding interface formed by NaV IQ motif sequences binding to calmodulin from multiple eukaryotes. NIH R01 GM57001.

2918-Pos Board B348
Coupling Compartmental Models to Live Neurons to Investigate Action Potential Mechanisms
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In mammalian central neurons, action potentials are initiated in the axonal initial segment (AIS) by Nav1.2 and Nav1.6 channels, and shaped and terminated by other voltage-gated ion channels. From the AIS, the AP travels down the axon towards the presynaptic site, but also back-propagates towards the soma. The role of axonal sodium channels in AP initiation and propagation is still incompletely understood, mostly because it is difficult to record from these channels at the AIS. Instead, most experimental evidence of axonal activity is obtained indirectly, from electrical recordings at the soma. To better understand these mechanisms, we developed a real-time computational procedure where a compartmental model of the axon is coupled to a live neuron using dynamic clamp. The properties of this computational model (e.g., spatial distribution and kinetics of ion channels) are varied until the firing activity of the hybrid construct (neuron + axon compartmental model) best matches the normal activity of the neuron.

2919-Pos Board B349
Optimizing a Nav1.5 Markov-Model with a Genetic Algorithm
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Background: Markov models of cardiac voltage-gated Na^+ (Na_V) channels have been widely used for cell and tissue simulations of cardiac electrophysiology. These $Na_V1.5$ models are of varying complexity. Simple models with few states cannot account for all of the kinetics observed with multiple protocols. Increasingly complex models become computationally infeasible for multi-scale simulations and their results are difficult to interpret.
Methods: Following previously published work for the neuronal Na^+ channel, we have implemented a genetic algorithm to optimize model topology and rate parameters for the cardiac sodium channel. The advantage of this approach is a flexible topology, with unnecessary states and edges removed in favor of model plausibility and computational speed. We improved this model by parallelizing the computation and including subpopulations with random mutation rates. Patch-clamp data for model parameterization was collected by recording transiently transfected HEK293T cells expressing $Na_v1.5$ channels 24 hours post-transfection. The temperature of the recordings was controlled at various temperatures ranging from room temperature to physiological temperatures. Models were successfully fit to patch-clamp protocols, consisting of activation curves, inactivation, recovery from inactivation, and activation current traces.
Results: The final optimization employed 32 subpopulations each with 50 members and was run for 2000 generations. After 2 x 10^6 models were evaluated, the algorithm yielded a model composed of 8 states and 10 interconnecting edges. The model was able to reproduce 20ms, 100ms, and 1000ms inactivation holds, activation, and recovery from inactivation protocols with high fidelity. Fast kinetic data was reproduced by fitting traces for −30mV, −10mV, and 10 mV directly. This novel model fits a wide range of experimentally collected data and contains significantly fewer parameters than current, widely used models.

2920-Pos Board B350
Rate Constant Models cannot Describe Movement of Charged Atoms or Molecules
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Rate constant models built on laws of mass action are used widely to describe movement of ions and electric current through channels, and chemical reactions of charged substrates. But mass action laws are derived from conservation of mass and say nothing about electric charge. Laws of electricity (Maxwell's equations: a generalization of Kirchoff's current law) are about conservation of charge, not mass. In classical rate models, flows of ions are not correlated by laws of electricity. But Maxwell's equations strongly correlate flows of charge (including displacement current), **without known exception**, within one part in 10^{18} or so. Correlation coefficients (describing correlated flows of charges in rate models) should then be nearly one, something like 0.999 999 999 999 999 999. Correlations of charge movement are ignored in classical rate models, so **classical rate models cannot describe movements of charged atoms or molecules with one set of rate constants over a range of conditions**.
A proof goes like this:
Consider the spatial series of reactions
X <==>**Y** <==>**Z**
Currents in a series of reactions analyzed by mass action are not (in general) equal:
$I_{XY}/F = z_X k_{XY}$ **[X]**-$z_Y k_{YX}$ **[Y]**; $I_{YZ}/F = z_Y k_{YZ}$**[Y]**-$z_Z k_{ZY}$**[Z]**
Kirchoff's current law requires $I_{XY} = I_{YZ}$ under all circumstances and conditions.
Details can be found at http://arxiv.org/abs/1409.0243 on the physics archive. The artifactual difference I_{XY} - I_{YZ} can have large effects**.** It can produce net charge and electric fields strong enough to break down membranes, proteins, chemical bonds, and even ionize atoms, because of the enormous strength of the electric field, as described unforgettably in p.1-1, of "***Feynman's Lectures on Physics, Vol. 2, Mainly Electromagnetism***…" http://www.feynmanlectures.caltech.edu/II_toc.html.

Voltage-gated Ca Channels

2921-Pos Board B351
Targeting T-Type Channels with Protxii-Like Toxins
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Few gating-modifier toxins have been reported to specifically target T-type calcium channels, and the structural basis of toxin sensitivity remains incompletely understood. Unlike the homotetrameric Kv channels, voltage-gated calcium channels are comprised of four different domains, presenting the possibility of multiple toxin binding sites. Studies of Kv channels identified a S3b-S4 helix-turn-helix motif, termed paddle motif, which moves at